How Does Reaction *vs* Diffusion Regulate ROS/RNS Biology?

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Two Properties Characteristic of Small, Uncharged, Highly Reactive Molecules

- Diffusibility
- Kinetics of Reaction

Diffusibility

- How Reactions Really Occur: Importance of Solvent Diffusion
- Net Movement of Collections of Molecules
- Sources and Sinks
- Spatial Confinement of NO
- Crowding in the Cytoplasm

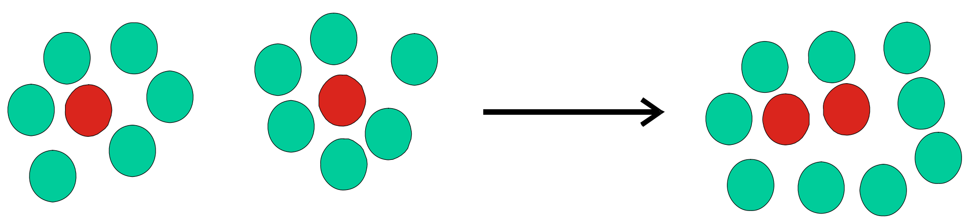
Kinetics of Reaction

- Rate *vs* Rate Constant
- Significance of zero order kinetics

How Reactions Really Occur

In order for two molecules to react, they must collide. However, of the billions of chemical reactions, only a handful are rapid enough that the reaction will occur after only a few collisions. Thus, virtually all reactions occur only after the two molecules collide in "just the right way."

In the gaseous phase, the distance between molecules is great enough to insure that molecules will separate rapidly after the collision. In the condensed phase, however (in aqueous solution or in tissue), individual molecules are surrounded by a "cage" of solvent (H_2O) molecules. Thus, parts of the "walls" of these cages must move away from two solutes (reactants) in order for collisions to occur:



Once this happens, however, the molecular pair is surrounded by the solvent cage which means that during the lifetime of the cage (which is 10^{-10} – 10^{-8} sec) the reactant molecules are colliding repeatedly. This is termed an "encounter."

If the probability of the reaction is high enough that the reaction will occur every time there is an encounter, then the rate of the reaction (the <u>overall</u> rate) will depend <u>only</u> on how fast the two molecules will form an encounter. This is called the "diffusion limit." No reaction can occur more rapidly than this limit.

This limit is in the range 10^{9}-10^{10} $M^{-1}s^{-1}$ (this is the value for k). Many reactions of radicals occur at this diffusion limit.

The Diffusion Constant: How Fast a Molecule Moves

Stokes' Law:

$$D = \frac{kT}{4\pi\eta r}$$

Temperature (kinetic energy)

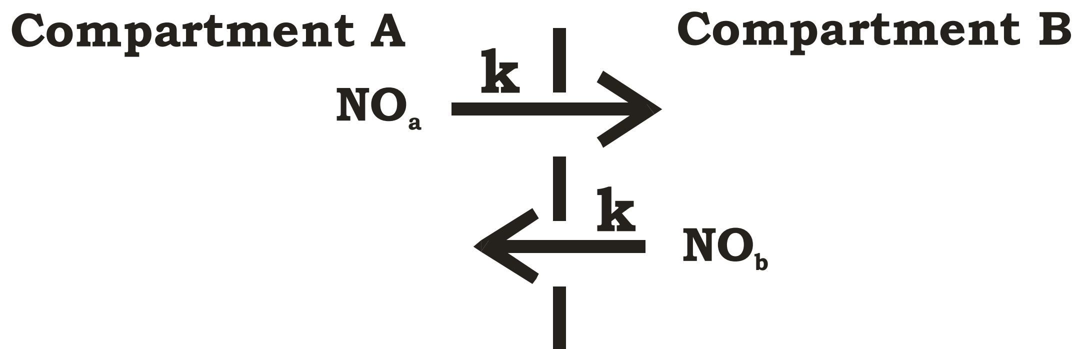
Viscosity (solvent mobility)

Solute size

For NO ($r = 1.4 \times 10^{-10}$ m)
in H_2O at 37° C,
D = 2740 μm²/s

	D (μm²/s)
NO (H_2O)	3300
NO (Brain)	3810
Ethanol	1100
Glucose	570
Sucrose	460
Myoglobin	113
Hemoglobin	69

Net Movement of Collections of Molecules



Probability for NO moving from A to B:
$k \times [NO]_a$

Probability for NO moving from B to A:
$k \times [NO]_b$

If $[NO]_a = [NO]_b$, No Net Movement
(NO's will just "change places")
If $[NO]_a > [NO]_b$, Net Movement from A to B
If $[NO]_b > [NO]_a$, Net Movement from B to A

CONCLUSION: The only thing that "makes" molecules move from one place to another is the presence of concentration gradients. Molecules will <u>always</u> move "downhill".

Theoretical Predictions of Diffusion in Aqueous Solution

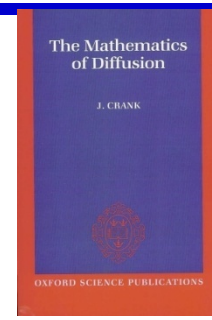
Method 1: Analytical Solutions

Method 2: Numerical Solutions

Analytical Solutions: based on Fick's Laws

$$F = -D\,\partial C/\partial x$$

The Bible: Crank "The Mathematics of Diffusion":



If the diffusing substance is initially distributed uniformly through a sphere of radius a, the concentration C at radius r, and time t is given by

$$C = \tfrac{1}{2}C_0\left\{\operatorname{erf}\frac{a-r}{2\sqrt{(Dt)}} + \operatorname{erf}\frac{a+r}{2\sqrt{(Dt)}}\right\} - \frac{C_0}{r}\sqrt{\left(\frac{Dt}{\pi}\right)}\left[\exp\{-(a-r)^2/4Dt\} - \exp\{-(a+r)^2/4Dt\}\right], \quad (3.8)$$

An Example: Diffusion away from a sphere:

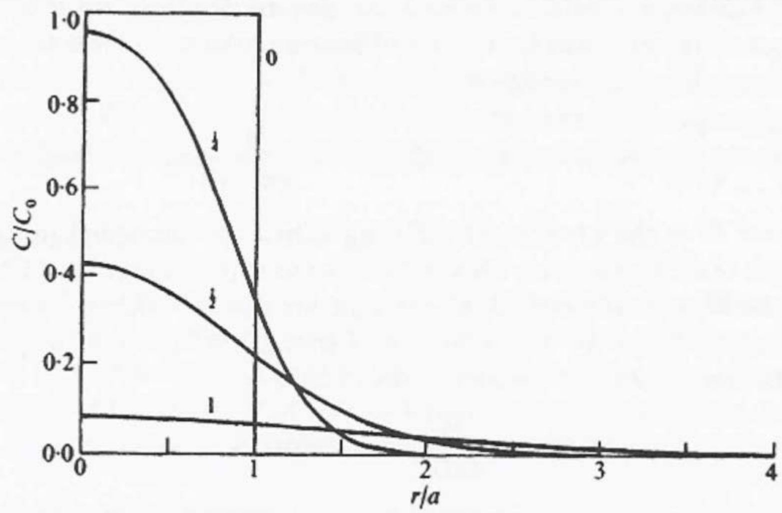
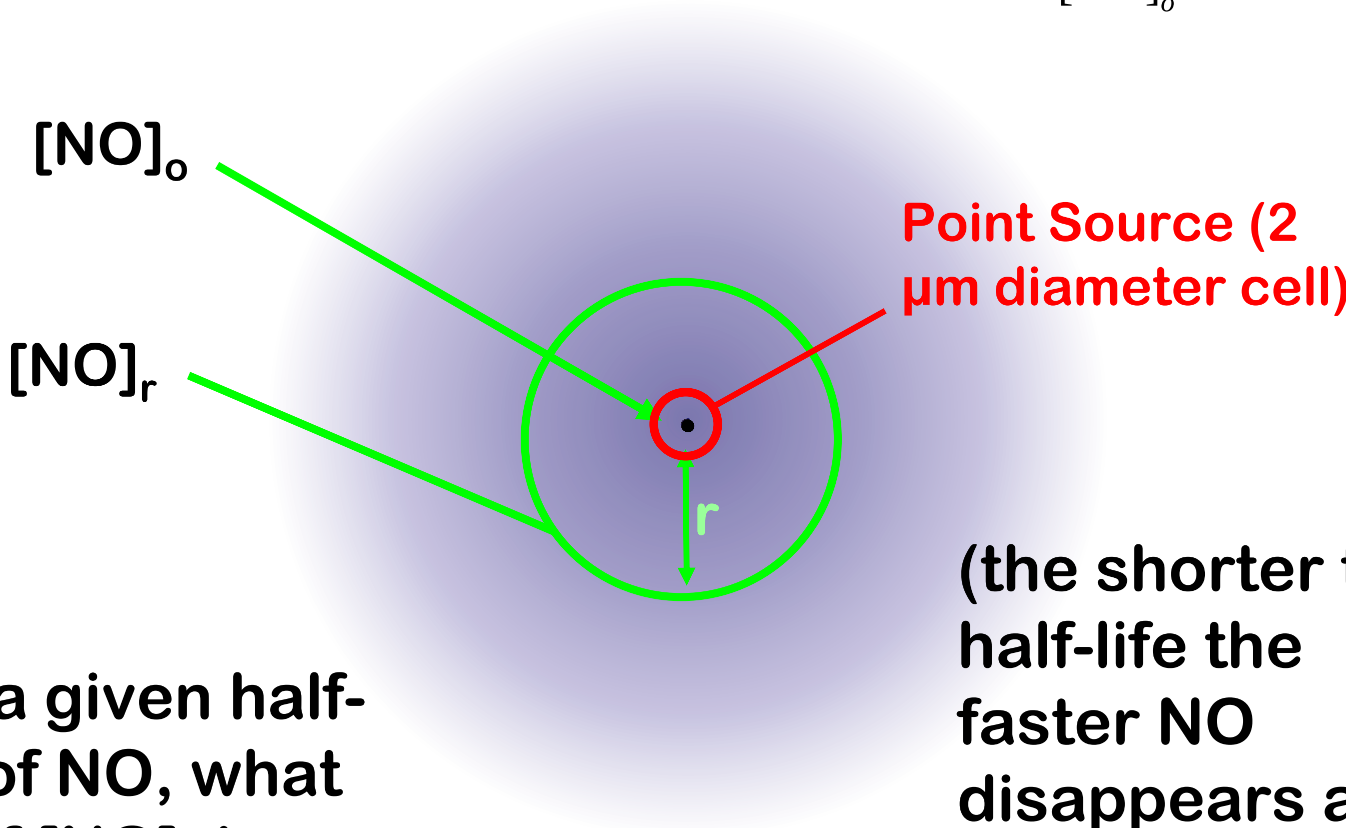


FIG. 3.1. Concentration distributions for a spherical source. Numbers on curves are values of $(Dt/a^2)^{\frac{1}{2}}$.

Steady-State: $\dfrac{\partial[NO]}{\partial t} = D\dfrac{\partial^2[NO]}{\partial r} - k[NO]$

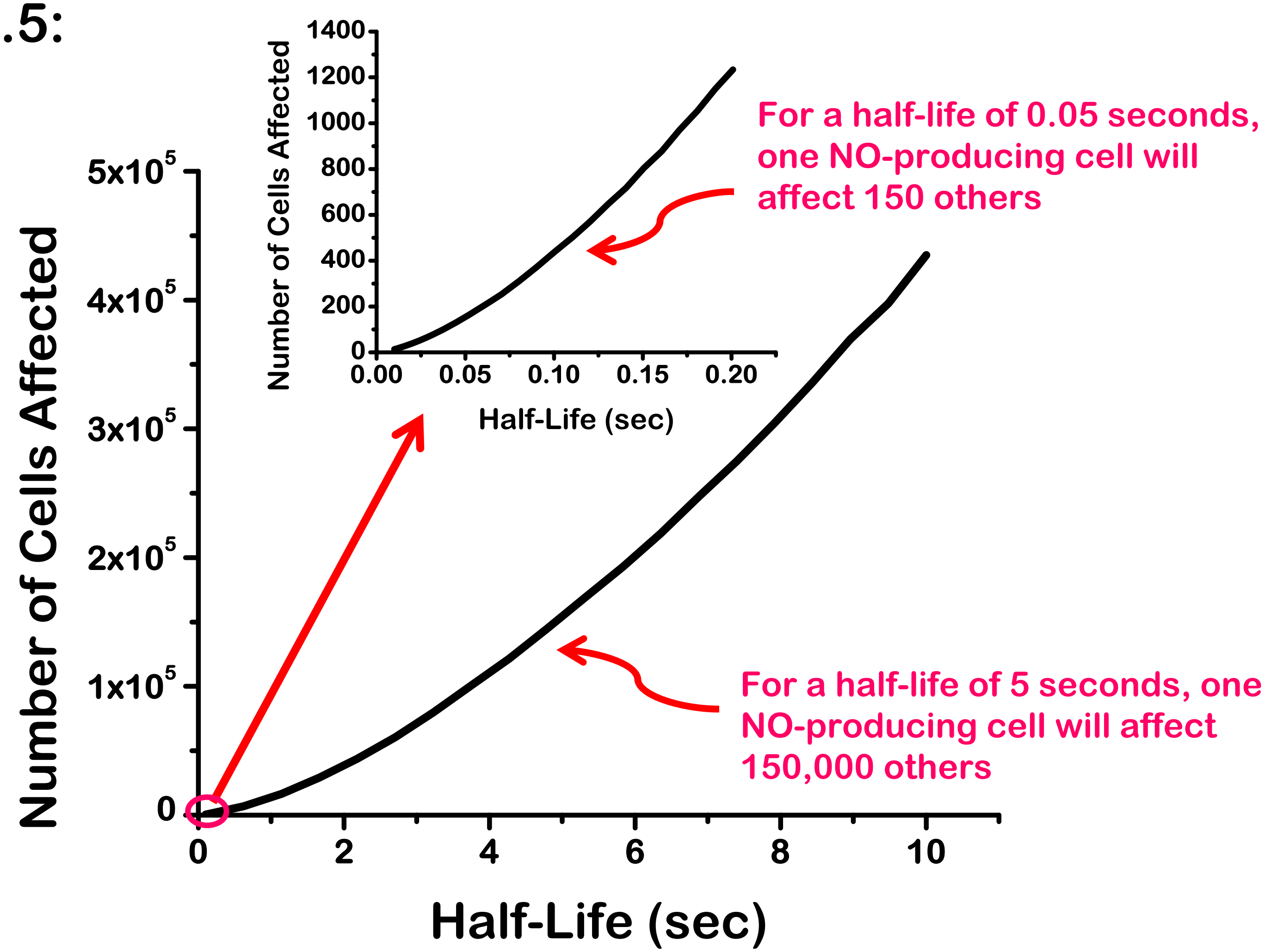
$$\frac{[NO]_r}{[NO]_o} = e^{-r\left(\frac{\ln 2}{D \times t_{1/2}}\right)}$$



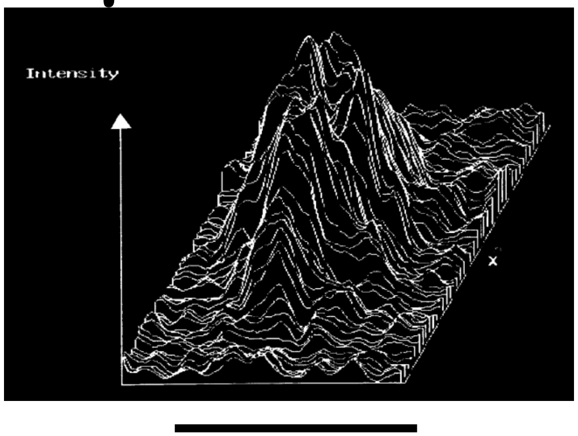
For a given half-life of NO, what is r if $[NO]_r$ is 50% of $[NO]_o$?

(the shorter the half-life the faster NO disappears as it travels, and so r will decrease)

For cells 2 μm diameter (3.35×10^{-14} L/cell); $[NO]_r/[NO]_o$ = 0.5:

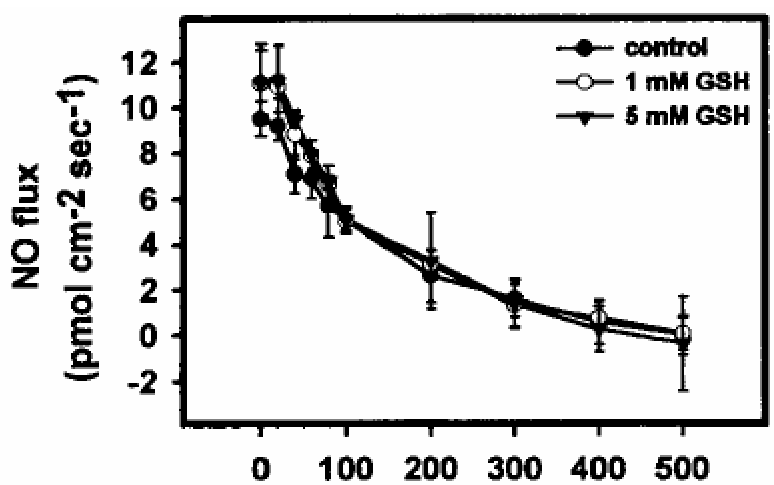


Experimental:

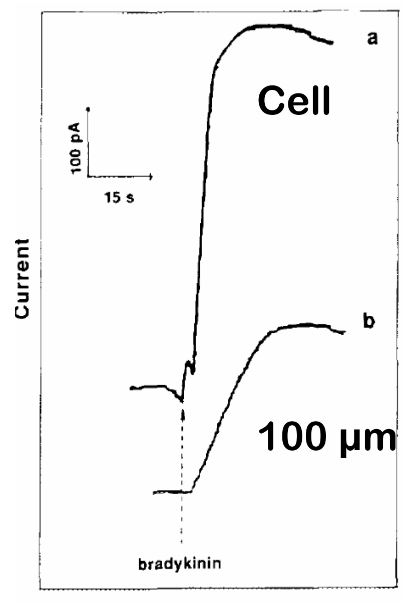


175 µm

Leone et al. BBRC 221:37 (96)

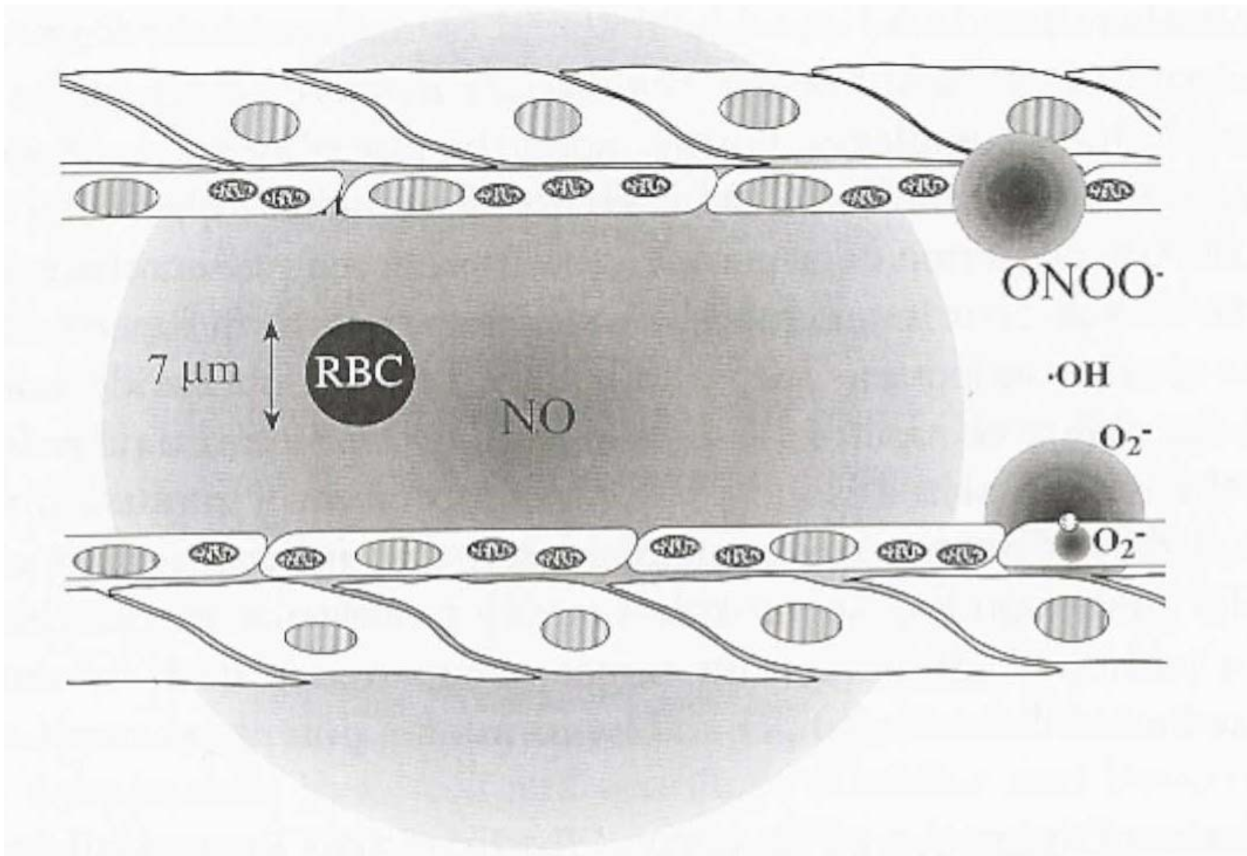


Porterfield et al. AJP 281:L904 (01)



Malinski et al. BBRC 193:1076 (93)

Comparison with Other Reactive Species:

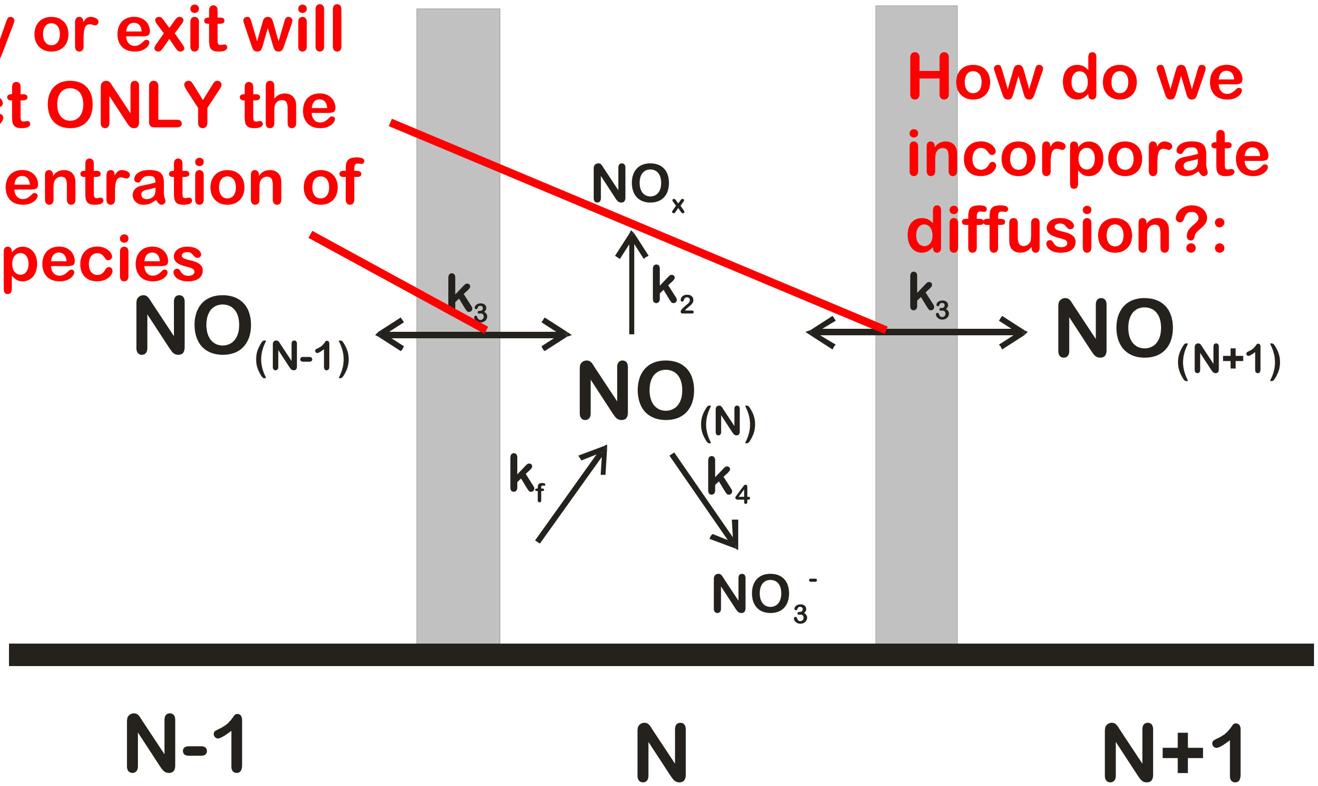


Beckman in “Nitric Oxide: Principles and Actions” Lancaster, ed. (1996)

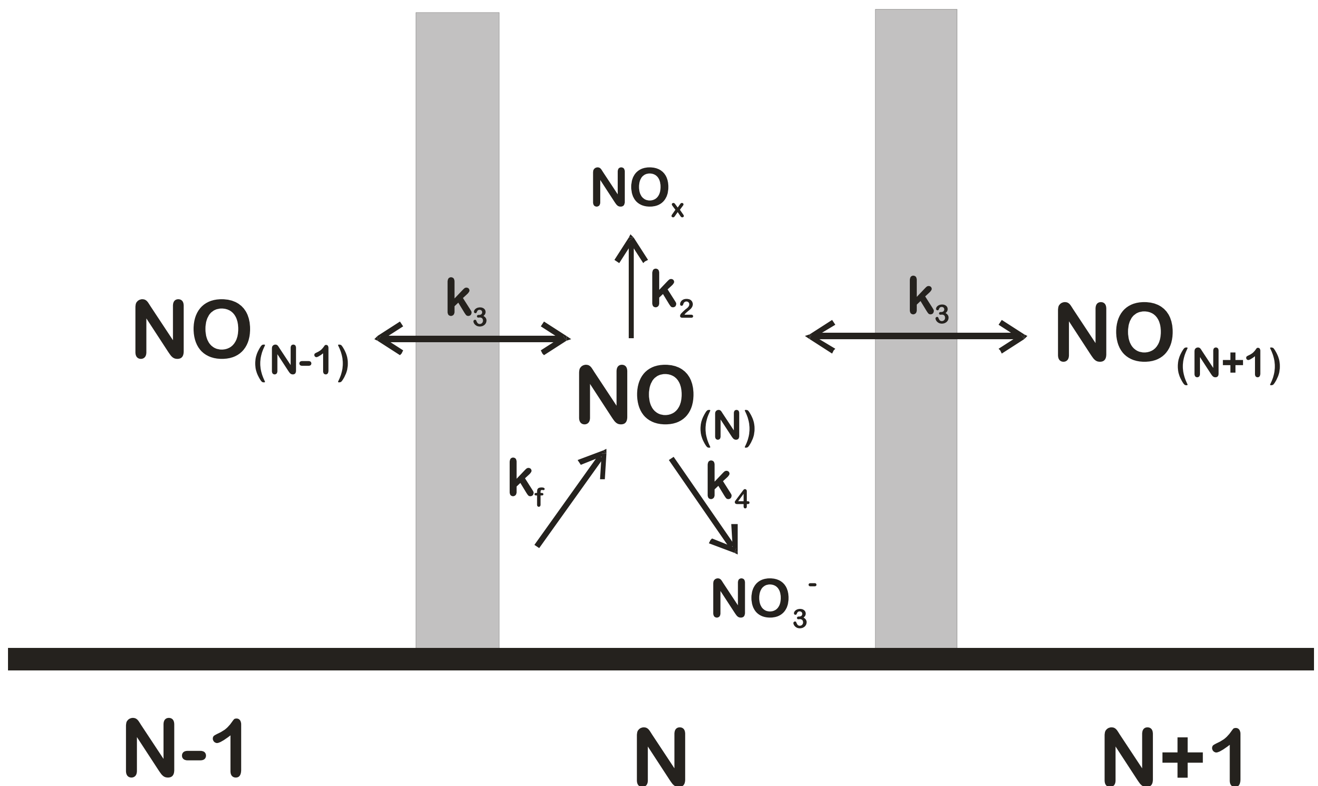
A Numerical Solution: Illustration of the Effect of Diffusion on Reaction:

Entry or exit will affect ONLY the concentration of the species

How do we incorporate diffusion?:



Lancaster Meth. Enz. 268:31(96)



$$\frac{d[NO]_{(N)}}{dt} = k_3[NO]_{(N-1)} + k_3[NO]_{(N+1)} - 2\times[NO]_{(N)} + k_f - (k_2 + k_4)[NO]_{(N)}$$

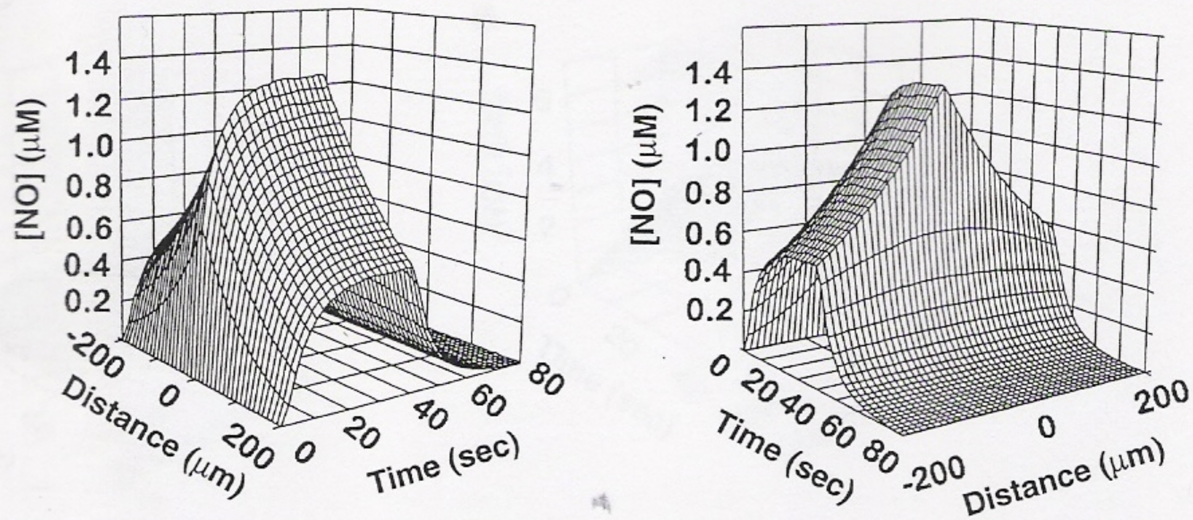
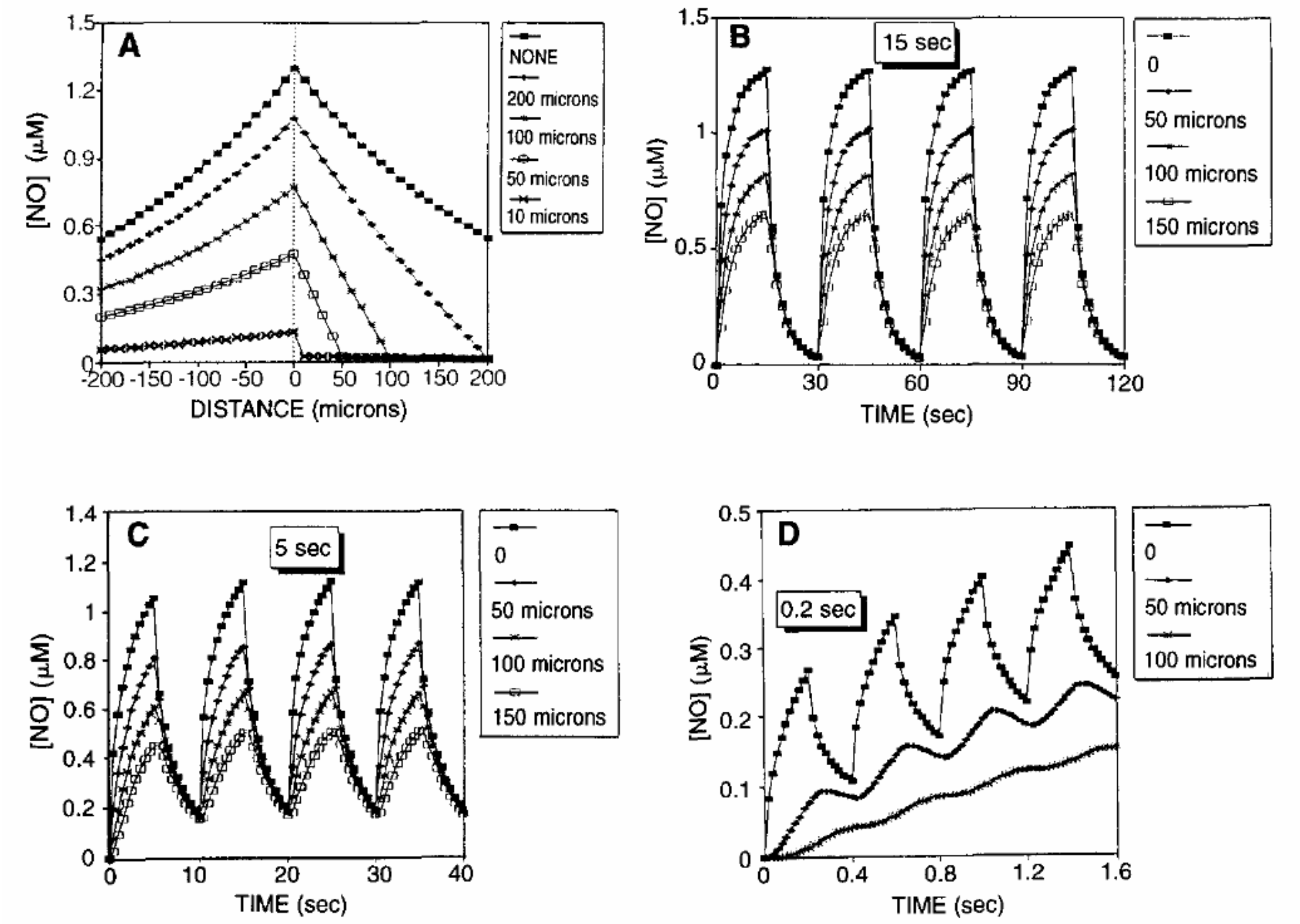


Figure 10 Representation of the diffusional spread of NO from a source, with initiation, steady state, and termination.

Lancaster in "Nitric Oxide Biology and Pathobiology" LJ Ignarro, ed. (00)

Sources and Sinks:



Lancaster Meth. Enz. 268:31(96)

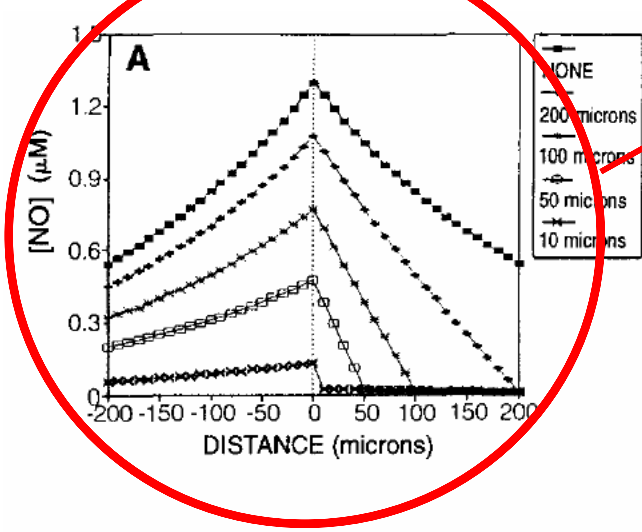
Spatial Confinement of NO

Spatial and Temporal Aspects of NO Signaling
In recent years, the notion that the transduction of signals relies on the free diffusion of molecules within the cell has been replaced by an appreciation that signaling takes place within the confines of subcellular compartments that are critical for both specificity of targeting and propagation of signals (Davare et al., 2001).

Stamler et al. Cell 106:675(01)

Here, we demonstrate that xanthine oxidoreductase (XOR), a prototypic superoxide $O_2^{\bullet -}$-producing enzyme, and neuronal nitric oxide synthase (NOS1) coimmunoprecipitate and colocalize in the sarcoplasmic reticulum of cardiac myocytes.

Khan et al. PNAS 101:15944(04)

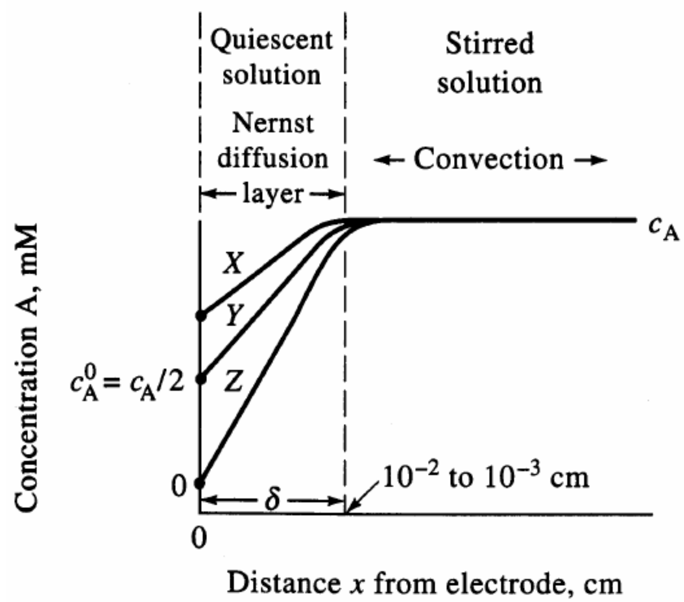


Only way to spatially confine NO: Change its r (reaction)

$$D = \frac{kT}{4\pi\eta r}$$

Is Measurement of D with Electrodes Truly Reflective of Diffusion in Cells?:

Polarographic Measurement of D:



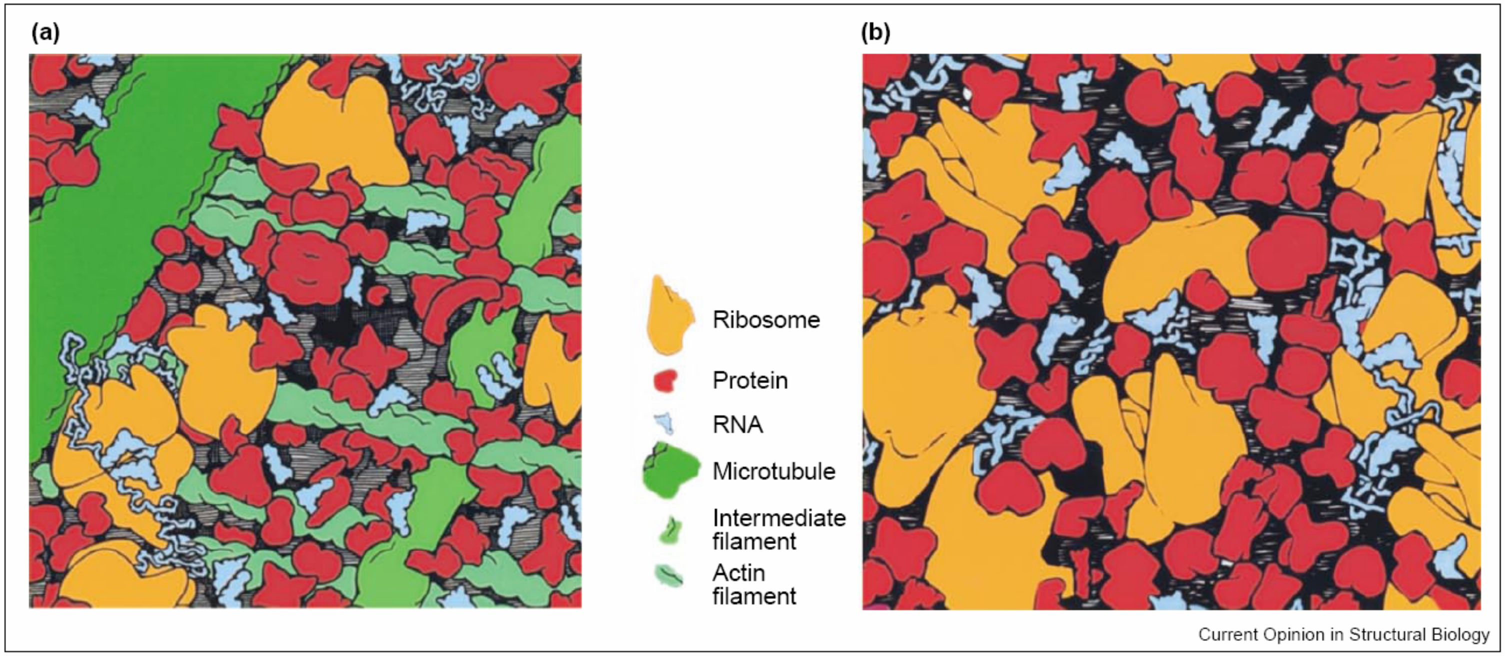
The Ilkovic Equation:

$$i = nFAD_A \underbrace{\left(\frac{\partial c_A}{\partial x}\right)}_{\text{rate of change of [A] with x}}$$

D_A only in the Nernst layer!

Crowding in the Cytoplasm

Figure 1



The crowded state of the cytoplasm in **(a)** eukaryotic and **(b)** *E. coli* cells. Each square illustrates the face of a cube of cytoplasm with an edge 100 nm in length. The sizes, shapes and numbers of macromolecules are approximately correct. Small molecules are not shown. Adapted with permission from [21].

“Macromolecular Crowding: an Important but neglected Aspect of the Intracellular Environment” Curr. Opin. Struct. Biol. 11:114(01)

Four factors that account for slowed diffusion:

-- Slowed diffusion in fluid-phase cytoplasm

-- Probe binding to intracellular components

-- Probe collisions with intracellular components (crowding)

Verkman “Solute and Macromolecule Diffusion in Cellular Aqueous Compartments” Trends Biochem Sci 27:27 (02)

What are the data?

Table 1

Diffusion of large and small molecules inside cells.

System	Molecular species	D/D_0*	References
Water	GFP	1	
CHO cell cytoplasm	GFP	0.31	[23]
CHO cell mitochondria	GFP	0.23–0.34	[24]
E. coli cytoplasm	GFP	0.088	[10•]
BSA (200 g/l)	FITC BSA	0.25	[25]
3T3 fibroblast cytoplasm	Carboxyfluorescein	0.27	[26]
3T3 fibroblast cytoplasm	FITC dextrans	0.27	[27]
Erythrocyte cytoplasm	Lactate	0.32	[28]

*Ratio of translational diffusion coefficient to that in water.

Kao *et al.* "Determinants of the Translational Mobility of a Small Solute in Cell Cytoplasm" J Cell Biol. 120:175 (93)

RATE VS. RATE CONSTANT:

Two determinants of how fast a reaction occurs:
Concentrations of reactants and rate constant:

$$A \xrightarrow{k} B$$

$$\frac{d[B]}{dt} = k[A]$$

Rate

Rate Constant

For many reactions involving radicals, rate constants (k) are very large:

Table S1. Elementary reactions and rate constants used in the kinetic simulations of product formation from peroxynitrite decomposition

entry	reaction[a]	k^{b} (s^{-1}; $M^{-1}s^{-1}$; $M^{-2}s^{-1}$)	refs	remarks
1	$O_2^{\bullet-} + NO^{\bullet} \rightarrow ONOO^-$	6.7×10^{9}	1	
2	$ONOO^- \rightarrow O_2^{\bullet-} + NO^{\bullet}$	1.7×10^{-2}	2	
3	$ONOO^- + H_3O^+ \rightarrow HOONO + H_2O$	5×10^{10}	3,4	estimated
4	$ONOOH + H_2O \rightarrow ONOO^- + H_3O^+$	1.43×10^{2}		from entry 3 and $pK_s = 6.8$
5	$ONOOH \rightarrow HNO_3$	0.94	5,6	72% of 1.3 s^{-1}, 37 °C
6	$ONOOH \rightarrow NO_2^{\bullet} + HO^{\bullet}$	0.36	7	28% of 1.3 s^{-1}, 37 °C
7	$ONOO^- + HO^{\bullet} \rightarrow O_2 + NO^{\bullet} + HO^-$	4.8×10^{9}	8	
8	$ONOOH + HO^{\bullet} \rightarrow O_2 + NO^{\bullet} + H_2O$	2×10^{7}		estimated, similar to H_2O_2, entry 11
9	$NO_2^- + HO^{\bullet} \rightarrow HO^- + NO_2^{\bullet}$	5.3×10^{9}	9	
10	$2\ HO^{\bullet} \rightarrow H_2O_2$	5.5×10^{9}	10	
11	$H_2O_2 + HO^{\bullet} \rightarrow HOO^{\bullet} + H_2O$	2.7×10^{7}	10	
12	$2\ HOO^{\bullet} \rightarrow H_2O_2 + O_2$	8.6×10^{5}	10	
13	$HOO^- + HO^{\bullet} \rightarrow HOO^{\bullet} + HO^-$	7.5×10^{9}	10	
14	$2\ O_2^{\bullet-} + H_2O \rightarrow O_2 + HOO^- + HO^-$	63	11	
15	$HOO^{\bullet} + O_2^{\bullet-} \rightarrow HOO^- + O_2$	9.7×10^{7}	12	
16	$HO^{\bullet} + NO^{\bullet} \rightarrow HNO_2$	1×10^{10}	13	
17	$HO^{\bullet} + NO_2^{\bullet} \rightarrow HNO_3$	4.5×10^{9}	14	
18	$HO^{\bullet} + NO_2^{\bullet} \rightarrow ONOOH$	4.5×10^{9}	14	from $k(17+18) = 1\times10^{10}$ $k(17)/k(18) = 1:1$
19	$HOO^{\bullet} + NO^{\bullet} \rightarrow ONOOH$	3.[illegible]	15	
20	$HOO^{\bullet} + NO_2^{\bullet} \rightarrow O_2NOOH$	4×10^{9}	16	
21	$O_2^{\bullet-} + NO_2^{\bullet} \rightarrow O_2NOO^-$	4.5[illegible]	16	
22	$H_2O_2 + H_2O \rightarrow HOO^- + H_3O^+$	3.[illegible]		from entry 23 and pK_s = 11.75
23	$HOO^- + H_3O^+ \rightarrow H_2O_2 + H_2O$	5×10^{10}	3,4	estimated
24	$H_3O^+ + NO_2^- \rightarrow HNO_2 + H_2O$	5×10^{10}	3,4	estimated
25	$HNO_2 + H_2O \rightarrow H_3O^+ + NO_2^-$	7.2[illegible]		from entry 24 and $pK_s = 3.1$
26	$H_3O^+ + O_2NOO^- \rightarrow O_2NOOH + H_2O$	5×10^{10}	3,4	estimated
27	$O_2NOOH + H_2O \rightarrow O_2NOO^- + H_3O^+$	1.43×10[illegible]		from entry 26 and $pK_s = 5.8$
28	$NO^{\bullet} + NO_2^{\bullet} \rightarrow N_2O_3$	1.1×[illegible]	17	
29	$N_2O_3 \rightarrow NO^{\bullet} + NO_2^{\bullet}$	8.0×10^{4}	17	

Table S1. continued

$\geq 10^9$

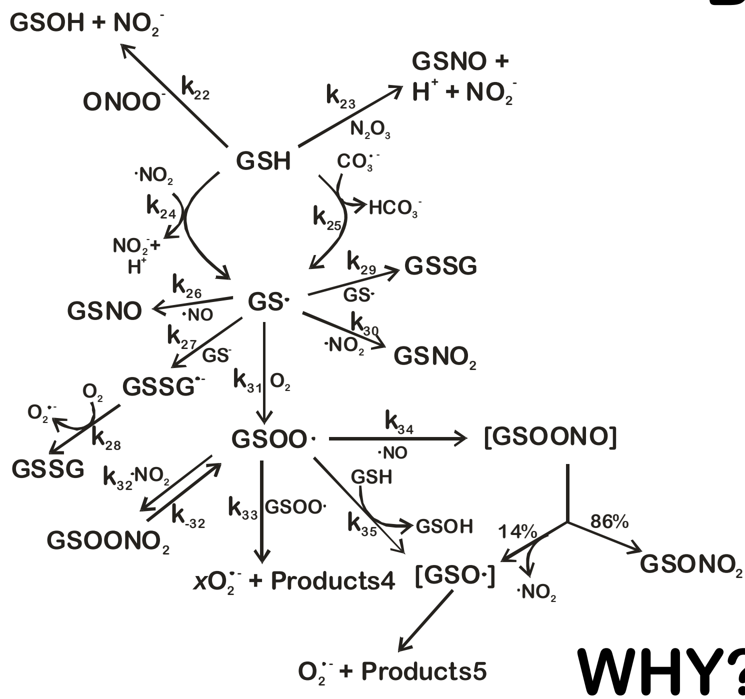
entry	reaction	k (s^{-1}; $M^{-1}s^{-1}$; $M^{-2}s^{-1}$)	refs	remarks
30	$2\ NO_2^{\bullet} \rightarrow N_2O_4$	4.5×10^{8}	14	
31	$N_2O_4 \rightarrow 2\ NO_2^{\bullet}$	6.9×10^{3}	17	
32	$HOO^{\bullet} + H_2O \rightarrow O_2^{\bullet-} + H_3O^+$	1.4×10^{4}		from entry 33 and $pK_s = 4.8$
33	$O_2^{\bullet-} + H_3O^+ \rightarrow HOO^{\bullet} + H_2O$	5×10^{10}	3,4	estimated
34	$O_2NOO^- \rightarrow NO_2^- + O_2$	1.4	18	
35	$O_2NOOH \rightarrow HNO_2 + O_2$	7.0×10^{-4}	16	
36	$O_2NOOH \rightarrow HOO^{\bullet} + NO_2^{\bullet}$	5×10^{-2}	19	
37	$N_2O_3 + H_2O\ (+\ OH^-) \rightarrow 2\ HNO_2$	$2\times10^{3}+10^{8}\times[OH^-]$	20	OH^--catalyzed
38	$N_2O_3 + H_2O\ (+\ HPO_4^{2-}) \rightarrow 2\ HNO_2$	$2\times10^{3}+8\times10^{5}\times[HPO_4^{2-}]$	21	HPO_4^{2-}-catalyzed
39	$2\ HNO_2 \rightarrow N_2O_3 + H_2O$	13.4	22	
40	$N_2O_4 + H_2O \rightarrow HNO_3 + HNO_2$	18	14,23	
41	$N_2O_3 + ONOO^- \rightarrow NO_2^- + 2\ NO_2^{\bullet}$	3.1×10^{8}	9,24	
42	$HNO_2 + O_2NOOH \rightarrow 2\ HNO_3$	12	25	
43	$HO^- + H_2O \rightarrow H_3O^+ + O^{\bullet-}$	1.1×10^{-3}		from entry 44 and $pK_s = 11.9$
44	$O^{\bullet-} + H_3O^+ \rightarrow HO^{\bullet} + H_2O$	5×10^{10}	3,4	
45	$NO^{\bullet} + ONOO^- \rightarrow NO_2^{\bullet} + NO_2^-$	5×10^{-2}	2	
46	$O^{\bullet-} + H_2O \rightarrow HO^{\bullet} + HO^-$	1.7×10^{6}	26	
47	$HO^{\bullet} + HO^- \rightarrow O^{\bullet-} + H_2O$	1.3×10^{10}	13	
48	$O^{\bullet-} + O_2 \rightarrow O_3^{\bullet-}$	3.8×10^{9}	27	
49	$O_3^{\bullet-} \rightarrow O^{\bullet-} + O_2$	4.0×10^{3}	28	
50	$O_3^{\bullet-} + O^{\bullet-} \rightarrow 2\ O_2^{\bullet-}$	7×10^{8}	29	
51	$HO^{\bullet} + O_3^{\bullet-} \rightarrow HOO^{\bullet} + O_2^{\bullet-}$	8.5×10^{9}	30	
52	$HO^{\bullet} + O_3^{\bullet-} \rightarrow HO^- + O_3$	2.5×10^{9}	28	
53	$O^{\bullet-} + O_2^{\bullet-} + H_2O \rightarrow O_2 + 2\ HO^-$	1.1×10^{7}	29	
54	$HO^{\bullet} + O_2^{\bullet-} \rightarrow O_2 + HO^-$	1.1×10^{10}	31	
55	$2\ O^{\bullet-} + H_2O \rightarrow HO^- + HOO^-$	4.5×10^{7}	13	
56	$O_3^{\bullet-} + O_2^{\bullet-} + H_2O \rightarrow 2\ HO^- + 2\ O_2$	5.0×10^{4}	32	
57	$O^{\bullet-} + NO^{\bullet} \rightarrow NO_2^-$	2×10^{9}		estimated
58	$O^{\bullet-} + NO_2^{\bullet} \rightarrow NO_3^-$	2×10^{9}	9	estimated

Table S1. continued

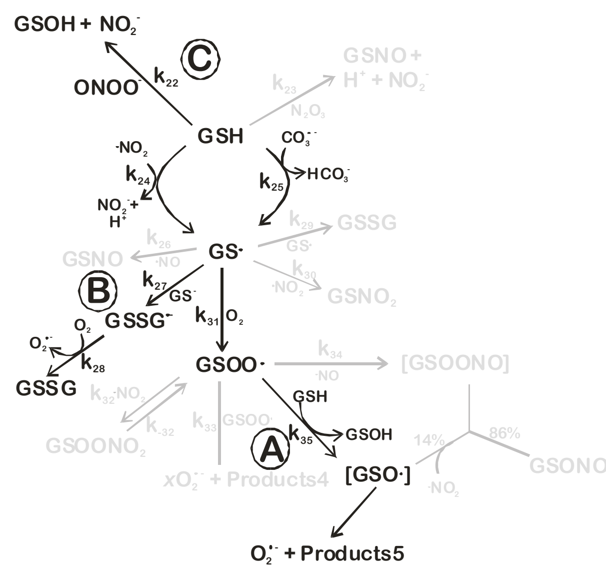
Kirsch et al. Chem. Eur. J. 7:3313 (01)

An example: Reactions of GSH with RNS

"All" Reactions:



Most Important Biologically:

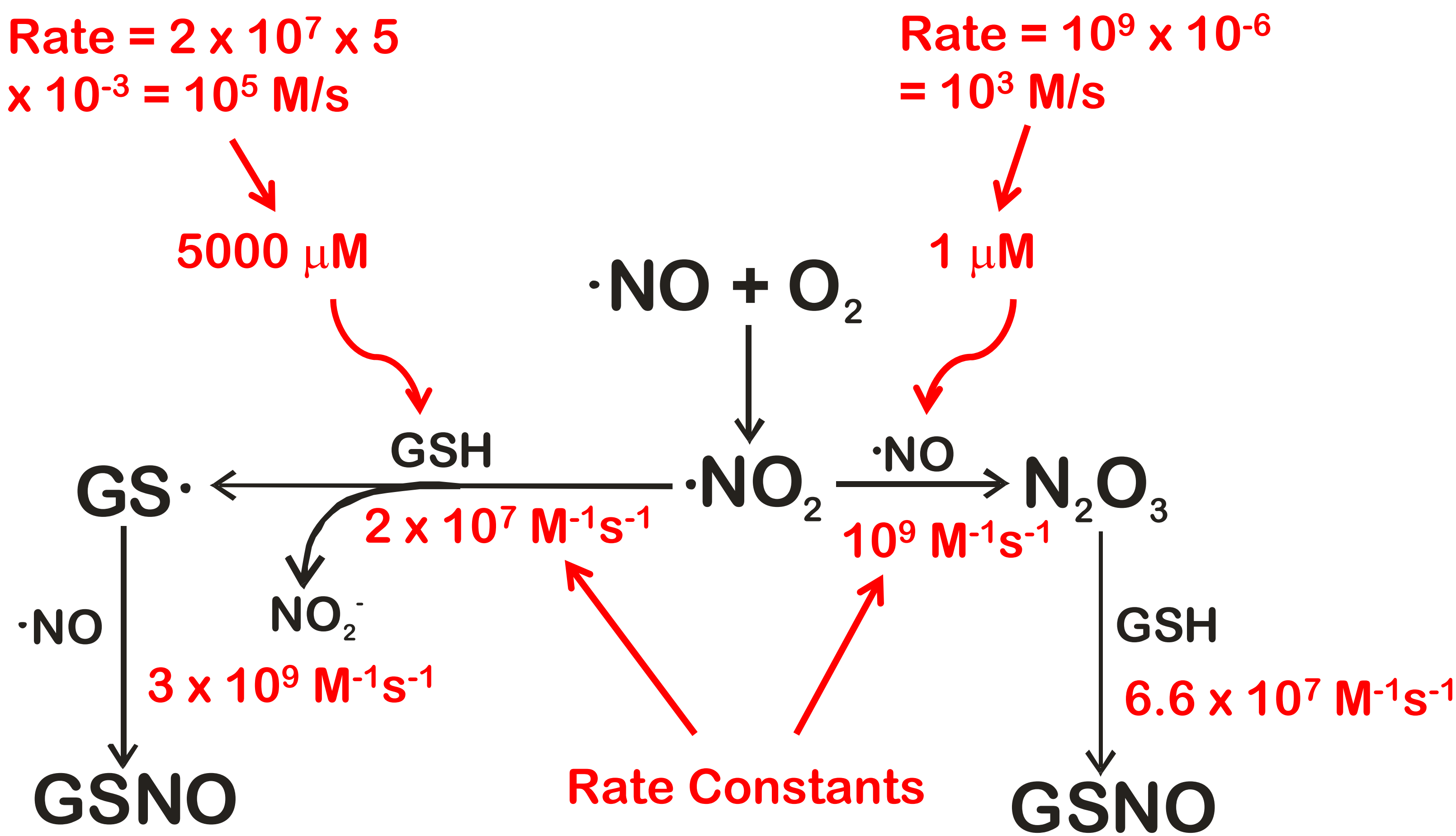


WHY?

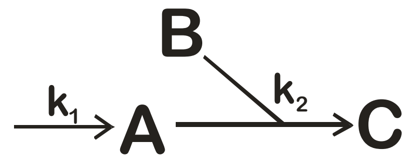
RATE VS. RATE CONSTANT

Lancaster Chem Res Toxicol 19:1160 (06)

RATE VS. RATE CONSTANT:



Significance of Zero Order Kinetics:



$$v = \frac{d[C]}{dt} = k_2[A][B]$$

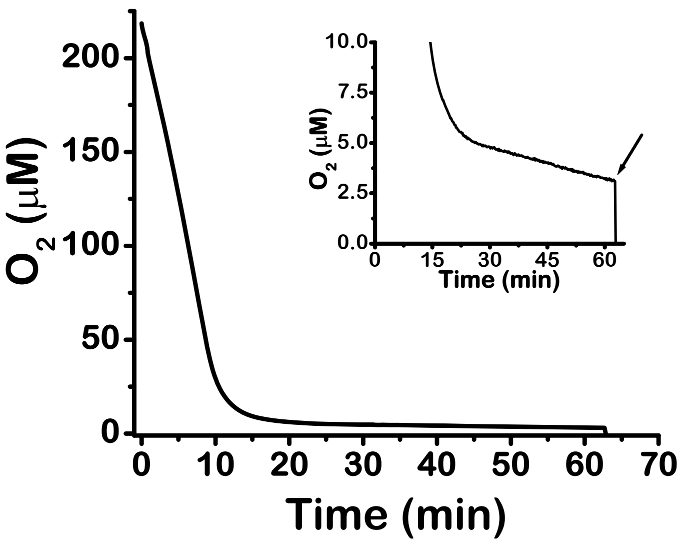
$$\frac{d[A]}{dt} = 0 = k_1 - k_2[A][B]$$

$$k_2[A][B] = k_1$$

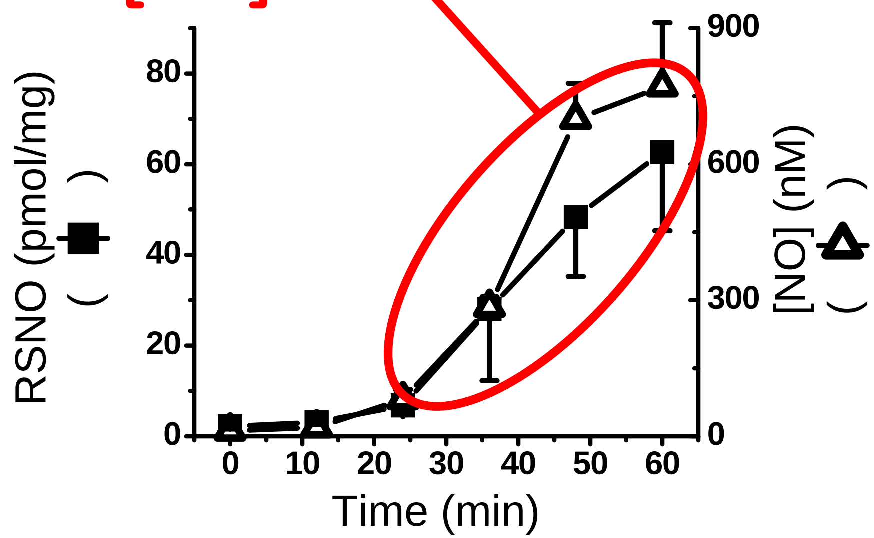
$$\therefore \; v = k_1$$

Rate of C formation is <u>independent</u> of concentration of B ("zero order" in [B]). Only determined by the rate of formation of A.

An Experimental Example: Measurement protein RSNO in cells treated with NO donor (Sper/NO); Simultaneous measurement of O_2 and NO:

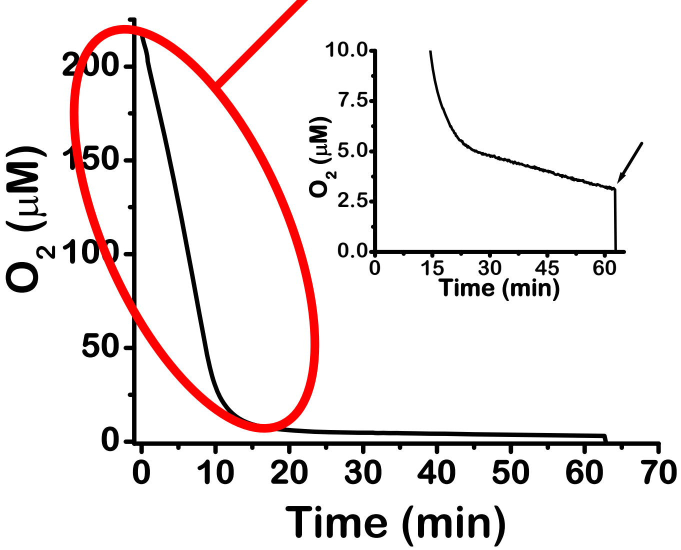


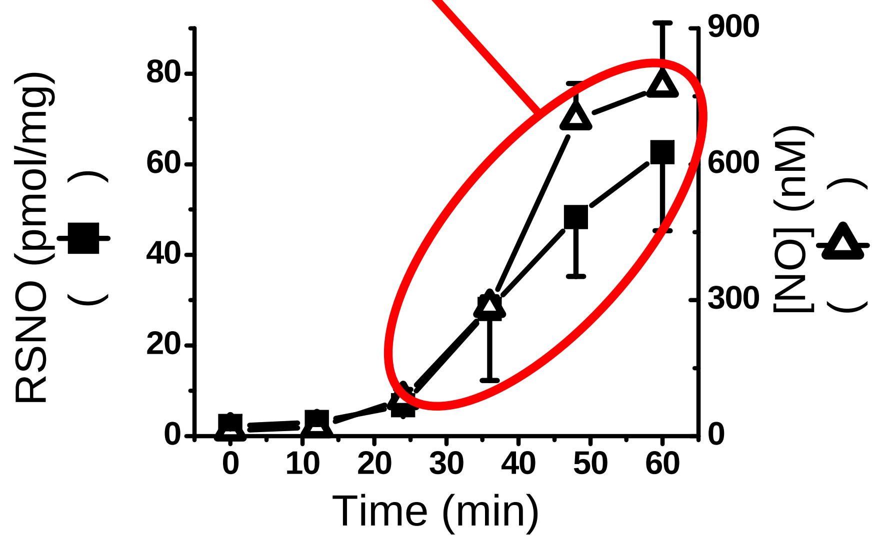
Rate of RSNO Formation is Zero Order in [NO]



An Experimental Example: Measurement protein RSNO in cells treated with NO donor (Sper/NO); Simultaneous measurement of O_2 and NO:

Also not dependent on $[O_2]$





Inconsistent with

$$2NO + O_2 \longrightarrow 2NO_2$$

$$NO + NO_2 \longrightarrow N_2O_3$$

$$N_2O_3 + RSH \longrightarrow RSNO + NO_2^- + H^+$$

Consistent with

$$\begin{array}{l} \big\downarrow \text{Rate Limiting} \\ RS\cdot + \cdot NO \longrightarrow RSNO \\ \big\downarrow O_2 \\ RSOO\cdot \end{array}$$

Charles Bosworth
Harry Mahtani
Jose Toledo
Bill Gates

American Cancer Society
NIH